DEVELOPMENT OF MOLECULAR IMPRINTED BIOSORBENT BY USING ORANGE PEEL FOR Pb^{2+} REMOVAL FROM AQUEOUS SOLUTION

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2010

ABSTRACT

In this research, the investigation of the use of orange peel which has high adsorption capacity of heavy metals compare to other types of biosorbent were carried out. In order to determine the most effective adsorption for each of heavy metals, we had come out in development of molecular-imprinted technology. Molecular-imprinted technique tends to give a higher adsorption rates and higher selectivity towards target metals. In order to achieve the objectives of this research, it would based on four main scopes of study which is to know effect of imprinting ions concentration on biosorption, effect of time on biosorption , effect of initial concentration of Pb^{2+} in solution and also to compare the different type of metals on adsorption selectivity of the biosorbent. The adsorption uptake was analyzed by using Atomic Absorption Spectrophotometer (AAS). In the preparation, with the imprinted ion Pb^{2+} concentrations increasing, the more fucntional groups (-OH) were protected and the more imprinting sites on the surface were retained. So, the loading amount of imprinted ions in preparation was 2 mg/g selected as the optimum loading amount. For the effect of time in adsorption, the optimum condition was described as the shortest time that the biosorbent can absorbed the metals. So, in this experiment, the shortest time that it can achieved with a high adsorption capacity was 30 minutes. It can be said as satisfactory adsorption compare to without imprinted biosorbent. The existence of functional group of (-OH) can be proved by using FTIR analysis. It was recommended that this research was furthered by using wastewater from metal industry, to recover back the Plumbum that had attached to the biosorbent, and can be applied in adsorption column.

ABSTRAK

Dalam kajian ini, satu penyiasatan tentang penggunaan kulit oren yang mempunyai kapasiti jerapan logam berat yang tinggi berbanding dengan jenis bio-penjerap yang lain telah di jalankan. Untuk menentukan jerapan yang paling berkesan untuk logam berat, kami telah keluar dengan pembangunan teknologi molekul-dicetak. Teknik Molekul-dicetak cenderung memberikan tahap jerapan tinggi dan selektivitas tinggi terhadap logam sasaran. Dalam rangka untuk mencapai tujuan kajian ini, ia akan berdasarkan kepada empat skop utama dalam kajian iaitu untuk mengetahui pengaruh mencetak kepekatan ion pada penjerapan, pengaruh waktu pada penjerapan, pengaruh kepekatan awal Pb^{2+} dalam larutan dan juga untuk membandingkan pelbagai jenis logam pada selektivitas jerapan daripada bio-penjerap. Penyerapan jerapan dianalisis dengan menggunakan Spektrofotometer Serapan Atom (SSA). Dalam masa persiapan, apabila kepekatan ion Pb^{2+} dicetak meningkat, lebih banyak kumpulan berfungsi (-OH) yang dilindungi dan lebih banyak laman percetakan di permukaan tetap dipertahankan. Jadi, jumlah pemuatan ion dicetak dalam persiapan adalah 2 mg / g dipilih sebagai jumlah optimum. Untuk pengaruh masa dalam jerapan, keadaan optimum digambarkan sebagai waktu terpendek untuk bio-penjerap dapat menyerap logam. Jadi, dalam percubaan ini, masa terpendek yang dapat dicapai dengan kapasiti jerapan tinggi adalah 30 minit. Hal ini dapat dikatakan sebagai jerapan yang amat memuaskan berbanding dengan bio-penjerap tanpa dicetak. Kewujudan kumpulan berfungsi (-OH) dapat dibuktikan dengan menggunakan analisis FTIR. Hal tersebut boleh dianjurkan untuk meneruskan kajian ini dengan menggunakan air sisa dari industri logam, untuk mendapatkan semula logam plumbum yang telah melekat pada bio-penjerap, dan boleh dilaksanakan dalam lajur jerapan.

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LIST OF ABBREVIATIONS

AAS	=	Atomic Absorption Spectrophotometer
EDTA	=	Ethylenediaminetetraacetic acid
Pb^{2+}	=	Plumbum ion
Nm^{-2}	=	Newton per metre square
FTIR	=	Fourier Transform Infrared
Ag (II)	=	Argentum
Ni (II)	=	Nickle
Cu (II)	=	Copper
Cd (II)	=	Cadmium
DOE	=	Department of Environment
OSHA	=	Occupational Safety and Health Administration
EPA	=	Environmental Protection Agency
µg /L	=	Microgram per litre
$\mu g/m^3$	=	Microgram per metre cube
mg/kg	=	Milligram per kilogram
µg /dL	=	Microgram per desilitre
mg/m^3	=	Milligram per metre cube
IQ	=	Intelligent Quotient
DHHS	=	Department of Health and Human Services
IARC	=	International Agency for Research on Cancer
NTP	=	National Toxicology Programme
CDC	=	Centers for Disease Control
RDA	=	Recommended Dietary Allowance
NIOSH	=	National Institute for Occupational Safety and Health
HDL	=	high-density lipoprotein
HM	=	heavy metals
HNO_3	=	Nitric Acid
NaOH	=	Sodium Hydroxide
-OH	=	Hydroxyl functional group
ppm	=	Part per million
C - C	=	Carbon-Carbon bond
STD	=	Standard

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Many industrial waste water effluents, particularly from mineral processing, metal plating; electric, electronic and chemical industries are environmentally unacceptably contaminated with heavy metal. Effects from these industrial activities may lead to heavy metal contamination in surface water, groundwater or even sea which then may cause toxic effects when they enter the food chain of the ecosystem. Traditional metal removal methods like chemical precipitation, chemical redox reactions, electrochemical treatment, membrane processes and ion exchange can be extremely expensive or inefficient, especially for large solution volumes at relatively very low concentrations (S.Schiewer *et al.*, 2008). The very common cationic heavy metals that may bring harmful to us are such as Pb (II), Ag (II), Ni (II), Cu (II) and also Cd (II). This type of metals had come out with some simple methods such as precipitation. This precipitation method uses some low cost alkaline materials such as lime to remove those heavy metals. However, this process usually produces large volumes of sludge consisting of small amounts of heavy metals in excess gypsum the recycling and reuse of which is very difficult (R.P.Dhakal *et al*., 2005).

Due to some drawback point of view from some of the methods, we had approach other methods that is by using biosorption. The efficient adsorption was based on the adsorbent itself. A large number of different adsorbent materials containing a variety of attached chemical functional groups has been reported for this purpose, with activated carbon being the most popular, however, the high cost of this material restricts its use on large scale (F.A.Pavan *et al*., 2006). In recent years, the natural adsorbent or biosorbent had been takes into account in order to replace activated carbon. The availability of this adsorbent had been a big advantage to use this type of adsorbent in order to remove heavy metals from industrial waste water. The potential type of biosorbent is from residuals of agricultural activities or wastes from food industries that are available in large amounts. One of the most efficient adsorbent was from fruit pectin. Pectin is the ionic plant polysaccharides, whose main structural features are the linear chains containing more than 100 (1-4)-linked α-D-galacturonic acid residue (Wong *et al*., 2008). Common types of pectin that we may use are such as orange pectin, apple pectin or durian rind pectin.

In this, the possibility of the use of citrus peel which is from orange peel which has high adsorption capacity of heavy metals compare to other types of biosorbent were investigated. In order to develop the most effective adsorption for targeted heavy metals, we enhance the performance of biosorbent by using molecular imprinting technique. By using surface molecular imprinting technology, this new biosorbent showed 30.0-50.0% higher uptake for Ni^{2+} in comparison to non-imprinted biosorbent (H.Huo *et al*., 2009). Besides, this type of technology had better mechanical performance and can be reused up to 15 cycles by producing the molecular templates of the heavy metals that are going to be removed.

1.2 Problem Statement

Industry which operates in heavy metals industry had introduced some heavy metals by discharging this waste into aquatic ecosystems. This problem had become a matter of concern over last few decades and had contributes to marine pollution. The pollutants of concern include silver, nickel, lead, chromium, zinc, cadmium, copper, gold and uranium. All these heavy metals bring harmful to ecosystems due to its toxicity. In order to give a better solution, the use of orange wastes from food industry that produces orange juices had been taking into account. Due to our latest technology, molecular imprinting biosorbent was prepared from orange pectin for better adsorption of heavy metals. Besides in helping to solve marine pollution which was polluted with toxic heavy metals, this research also count for the use of waste for other significant study.

Environmental contamination and exposure to heavy metals such as mercury, cadmium and lead is a serious growing problem throughout the world. Human exposure to heavy metals has risen dramatically in the last 50 years as a result of an exponential increase in the use of heavy metals in industrial processes and products. Many occupations involve daily heavy metal exposure; over 50 professions entail exposure to mercury alone. In today's industrial society, there is no escaping exposure to toxic chemicals and metals. In the United States, tons of toxic industrial waste are mixed with liquid agricultural fertilizers and dispersed across America's farmlands. This "controversial practice," which is presently legal in the U.S., has been reported in nine states. While the spreading of arsenic, lead, cadmium, nickel, mercury and uranium on soil that is utilized to produce food for human consumption is a "political and economic issue," the potential for adverse health effects is well documented.

1.3 Objectives

In this research study, the main objectives are to determine the formulation molecular imprinted based biosorbent and also to study and optimize the performance of imprinted biosorbent for heavy metal removal.

1.4 Scope of study

In order to achieve the objectives of this research, it would based on four main scopes of study which is to know effect of imprinting ions concentration on biosorption, effect of time on biosorption , effect of initial concentration of Pb^{2+} in solution and also to compare the different type of metals on adsorption selectivity of the biosorbent.

CHAPTER 2

LITERATURE REVIEW

2.1 Adsorption

The existence of industrial which involves in mineral processing, metal plating; electric, electronic and chemical industries had gives a big impact to our ecosystem and environment. These type of activities had contributes to heavy metal pollutions. In order to remove the pollutants or heavy metals, a number of methods were currently used. Some of them were chemical precipitation, chemical redox reactions, electrochemical treatment, membrane processes and ion exchange. But this method was extremely expensive and inefficient. However, there is another method that may only involve low cost usage, which is adsorption. Adsorption is one of the effective techniques for removal of heavy metal (M.Khormaei *et al*., 2007). Adsorption is a surface phenomenon and should not be confused with absorption, which refers to the penetration of substances into the porous structure within solid material Adsorption and ion exchange processes are the most useful methods to removal them, by exploring the availability of different kinds of adsorbents associated with convenient procedures for obtaining high efficiency (F.A.Pavan *et al*., 2006). Adsorption can be used to separate a molecule from a complex mixture of molecules, or simply to separate a solute from its solvent. This is achieved by contacting the solution with the solid material which is also called the adsorbent. The molecule that binds on the adsorbent is referred to as the adsorbate.

2.1.1 Biosorption

Biosorption is also one of adsorption process which involves the using of biomass as their biosorbent. Biosorption is the term given to the passive sorption and/or complexation of metal ions by biomass. The mechanisms of biosorption are generally based on physico-chemical interactions between metal ions and the functional groups present on the cell surface, such as electrostatic interactions, ion exchange and metal ion chelation or complexation (C.Mack *et al*., 2007). Biosorption was really effective and involves low cost for the wastewater treatment especially for heavy metal removal. Considering the viewpoint of sustainable development and comprehensive utilization of resources, biosorption has a promising prospect and a wide application due to its low cost, abundance and good performance over other conventional treatment processes in the removal and recovery of heavy metal ions from wastewater (H.Su *et al*., 2006). Biosorption also has some advantages compare to other methods to remove heavy metals. These include low operating costs, minimization of the volume of chemical and/or biological sludge to be handled and high efficiency in detoxifying effluents (F.A.Pavan *et al*., 2006).

2.2 Adsorbent

Most adsorption processes utilize particulate adsorbents. Most of the adsorbents are made from natural or synthetic material. Commonly used adsorbents in bioseparation processes include cellulose based adsorbents, silica gel, synthetic resins; agarose based adsorbents and cross linking dextran based adsorbents. However, this type of adsorbents was very expensive. In order to minimize the cost of water treatment, low cost adsorbents will be use to remove heavy metals, dyes and others. The use of biosorbents was one of the alternative ways to replace other adsorbents. The local availability was frequently from the residues of agricultural activity, food industry, or seafood processing. A potential cheap natural source is the abundant waste from the non-profitable part of fruits that might be useful for such procedure (C.Mack *et al.*, 2007). Some examples of biosorbent are such as banana peel, durian rind pectin (Wong *et al.*, 2008), orange pectin (R.P.Dhakal *et al.*, 2005, M.Khormaei *et al.*, 2007), chitosan (H.Su *et al.*, 2006), Ponkan mandarin peels (F.A.Pavan *et al.*, 2006), Yellow passion-fruit shell (F.A.Pavan *et al.*, 2006) and many others. Each of this biosorbent has high efficiency to remove heavy metals. Chitosan is one of the effective biosorbent. As a new kind of biosorbent, it has been prepared into different forms and widely used in the wastewater treatment because of its higher adsorption capacity and better selectivity for heavy metal ions. However, its application is limited because of its dissolution in acidic solutions and higher cost (H.Su *et al.*, 2006). Then, some of the researcher gives more focus on the using of fruits pectin which is the pectin was prepared from fruits peel and was forms in gel-like form. The selectivity of these gels of alginic acid and pectic acid which show remarkable separation features for heavy metal ions (R.P.Dhakal *et al.*, 2005).

2.3 Pectin

The texture of fruits and vegetables during growth, ripening and storage depends on the quantity and quality of pectin present.Pectin are the ionic plant polysaccharides, whose main structural features are the linear chains containing more than 100 (1-4)-linked α-D-galacturonic acid residue (Wong *et al.*, 2008). Part of the carboxyl groups of the anhydro-galacturonic acid is esterified with methanol. Other than free carboxyl groups, pectin also possess methylated ester groups in its polymeric chain. Such methyl ester groups were saponified with saturated calcium hydroxide solution to convert them into carboxyl groups according to the following reaction.

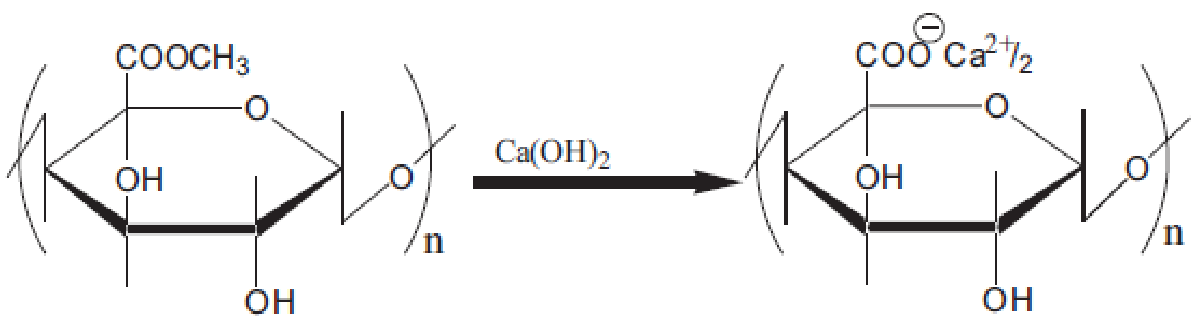

Figure 1 Methyl ester groups are saponified with saturated calcium hydroxide solution to convert them into carboxyl groups

The common types of pectin are such as citrus pectin, apple pectin, grapefruits and many others. Citrus Pectin is a complex polysaccharide obtained from the peel or pulp of the citrus fruits such as oranges. Citrus pectin has higher adsorption capacity rather than durian rind pectin (Wong *et al*., 2008).

2.4 Molecular Imprinting

Molecular imprinting is a technique to create template-shaped cavities in polymer matrices with memory of the template molecules to be used in molecular recognition. This technique is based on the system used by enzymes for substrate recognition, which is called the "lock and key" model. The active binding site of an enzyme has a unique geometric structure that is particularly suitable for a substrate. A substrate that has a corresponding shape to the site is recognized by selectively binding to the enzyme, while an incorrectly shaped molecule that does not fit the binding site is not recognized.

In a similar way, molecular imprinted materials are prepared using a template molecule and functional monomers that assemble around the template and subsequently get crosslinked to each other. The functional monomers, which are self-assembled around the template molecule by interaction between functional groups on both the template and monomers, are polymerized to form an imprinted matrix (commonly known in the scientific community as a molecularly imprinted polymer). Then the template molecule is removed from the matrix under certain conditions, leaving behind a cavity complementary in size and shape to the template. The obtained cavity can work as a selective binding site for a specific template molecule. This technique has very high selectivity. By using surface molecular imprinting technology, this new biosorbent showed 30.0-50.0% higher uptake for Ni^{2+} in comparison to non-imprinted biosorbents (H.Huo *et al*., 2009). In addition, it had better mechanical performance and could be reused for up to 15 cycles.

2.5 Heavy Metals

The disposal of heavy metals into aquatic streams has been the major concern to our worldwide over last few decades. This heavy metals can be defined as a group of element between copper and lead in the periodic table of the element having atomic weight between 63.546 and 200.59 and specific gravities greater than 4.0. Living organisms require trace amount of some heavy metals including cobalt, copper, molybdenum, vanadium, strontium and zinc but excessive levels can be detrimental to the organism. However, some procedures are introduced to remove heavy metals. The commonly used procedures for removing metal ions from dilute aqueous streams include chemical precipitation, reverse osmosis and solvent extraction (K.C.Sekhar *et al*., 2003).

Heavy metals are dangerous because they tend to bioaccumulates. Bioaccumulation means an increase in the concentration of a chemical in a biological organism over time, compared to the chemical's concentration in the environment. Bioaccumulation occurs when an organism absorbs a toxic substance at a rate greater than that at which the substance is lost. Thus, the longer the biological half-life of the substance the greater the risk of chronic poisoning, even if environmental levels of the toxin are not very high.

Heavy metals also can enter a water supply by industrial and consumer waste, or even from acid rain which then will flow into the soils and releasing the heavy metals into water streams, lakes, rivers and also groundwater. Table 1 have shown the limited parameter that has been used in Department of Environment (DOE). All the industry that use, produced or disposed heavy metals need to follow the standard before released it to the river or lakes.

Table 1 Parameter Limit for Standard A and Standard B

Parameter	Unit	Standard A	Standard B
Plumbum (II)	**mg/l**	**0.10**	**0.50**
Cadmium(II)	**mg/l**	**0.01**	**0.02**
Mangan(II)	**mg/l**	**0.20**	**1.00**
Nickle(II)	**mg/l**	**0.20**	**1.00**
Zinc(II)	**mg/l**	**2.00**	**2.00**
Ferum(II)	**mg/l**	**1.00**	**5.00**

2.5.1 Definition of Heavy Metal

Heavy metals are chemical elements with a specific gravity that is at least 5 times the specific gravity of water. The specific gravity of water is 1 at 4°C (39°F). Simply stated, specific gravity is a measure of density of a given amount of a solid substance when it is compared to an equal amount of water. Some well known toxic metallic elements with a specific gravity that is 5 or more times that of water are arsenic, 5.7; cadmium, 8.65; iron, 7.9;lead, 11.34; and 13.456;mercury.

2.5.2 Toxic Heavy Metal

There are more than 20 heavy metals, but four are of particular concern to human health: lead (Pb), cadmium (Cd), mercury (Hg), and inorganic arsenic (As). According to the U.S. Agency for Toxic Substances and Disease Registry, these four heavy metals are four of the top six hazards present in toxic waste sites. They are highly toxic and can cause damaging effects even at very low concentrations. They tend to accumulate in the food chain and in the body and can be stored in soft (e.g., kidney) and hard tissues (e.g., bone). Being metals, they often exist in a positively-charged form and can bind on to negatively-charged organic molecules to form complexes.

Heavy metals become toxic when they are not metabolized by the body and accumulate in the soft tissues. Heavy metals may enter the human body through food, water, air, or absorption through the skin when they come in contact with humans in agriculture and in manufacturing, pharmaceutical, industrial, or residential settings. Industrial exposure accounts for a common routes of exposures for adults. Ingestion is the most common routes of exposure for children. Children may develop toxic levels from the normal hand-to-mouth activity of small children who come in contact with contaminated soil.

The body has need for approximately 70 friendly trace element heavy metals, but there are another 12 poisonous heavy metals, such as Lead, Mercury, Aluminum, Arsenic, Cadmium, Nickel, etc., that act as poisonous interference to the enzyme systems and metabolism of the body. No matter how many good health supplements or procedures one takes, heavy metal overload will be a detriment to the natural healing functions of the body. Some metals are naturally found in the body and are essential to human health. Iron, for example, prevents anemia, and zinc is a cofactor in over 100 enzyme reactions. Magnesium and copper are other familiar metals that, in minute amounts, are necessary for proper metabolism to occur. They normally occur at low concentrations and are known as trace metals; for example, high levels of zinc can result in a deficiency of copper, another metal required by the body. Heavy or toxic metals are trace metals that are at least five times denser than water. As such, they are stable elements (meaning they cannot be metabolized by the body) and *bio-accumulative* (passed up the food chain to humans). These include: mercury, nickel, lead, arsenic, cadmium, aluminum, platinum, and copper (metallic form versus ionic form). Toxic heavy metals have no function in the body and can be highly toxic. Heavy metals are taken into the body via inhalation, ingestion, and skin absorption. If heavy metals enter and accumulate in body tissue faster than the body's detoxification pathways can dispose of them, a gradual buildup of these toxins will occur. High-concentration exposure is not necessary to produce a state of toxicity in the body tissues and, over time, can reach toxic concentration levels.

2.5.3 Plumbum

Lead is a naturally occurring bluish-gray metal found in small amounts in the earth's crust. It has no special taste or smell. It can be found in all parts of our environment. Most of it came from human activities like mining, manufacturing, and the burning of fossil fuels. Lead is used as a construction material for equipment used in sulfuric acid manufacture, petrol refining, halogenation,